

Stem cells: the generation and maintenance of cellular diversity

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Key words: stem cells, differentiation, growth factors, extracellular matrix.

Introduction

Cell divisions during embryonic development give rise to new differentiated cell types or increase the total number of cells in the embryo. In contrast, the major role of cell division in adult life is to maintain the number of differentiated cells at a constant level: to replace cells that have died or been lost through injury.

The rate at which new cells are produced in the adult is a measure of how rapidly the cell population is turning over and, on this basis, tissues can be divided into three broad categories (Leblond, 1963). In tissues with static cell populations, such as nerve and skeletal muscle, there is no cell division and most of the cells formed during development persist throughout adult life. In tissues containing conditional renewal populations, such as liver, there is generally little cell division, but in response to an appropriate stimulus most cells can divide to produce daughters of the same differentiated phenotype. Finally, tissues with permanently renewing populations, including blood, testis and stratified squamous epithelia, are characterized by rapid and continuous cell turnover in the adult: the terminally differentiated cells have a short lifespan and are replaced through proliferation of a distinct subpopulation of cells, known as stem cells.

The simplest definition of a stem cell is that it is any cell with a high capacity for self-renewal, extending

throughout adult life. In addition, stem cells are usually considered to have the potential to produce differentiated progeny and, as such, a stem cell may have a less 'mature' or less 'differentiated' phenotype than its daughters (Lajtha, 1979). Using these criteria, most of the progenitor cell populations that arise during embryonic development are not stem cells, since they do not self-renew; however, as we shall describe, they have a number of properties in common with the stem cells of adult organisms, including differentiation potential and capacity for asymmetric cell division.

Three adult mammalian tissues in which stem cells have been extensively studied are the haemopoietic system, the epidermis and intestinal epithelium. In our review, we shall begin by describing these stem cell populations: the evidence that they exist; their identification, location and differentiated progeny. Three common features that emerge are illustrated in Fig. 1A. First, stem cells have the capacity for unlimited self-renewal, in the context of the lifespan of the organism. Second, they have the potential for asymmetric divisions, such that one daughter is itself a stem cell while the other is committed to undergo terminal differentiation. Third, the differentiation process is irreversible, since the daughters of a cell that is committed to terminal differentiation are never stem cells.

In haemopoiesis and in intestinal epithelium, the stem cells are pluripotent, giving rise to more than



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one type of differentiated cell. In the epidermis, in contrast, there appears to be only a single pathway of terminal differentiation (Fig. 1B). In all three tissues, there is evidence that differentiation of stem cell progeny occurs *via* transit amplifying, or progenitor, populations that have a more limited capacity for self-renewal (Fig. 1B).

We shall discuss what is known of the mechanisms that regulate these aspects of stem cell behaviour, drawing on the properties of analogous cells in a range of other tissues and organisms.

Stem cells in adult self-renewing tissues

Haemopoiesis

The terminally differentiated cell types of blood, shown in Fig. 2, are all derived from pluripotent stem cells that are located in bone marrow. The first evidence for the existence of such a stem cell population came from experiments in which bone marrow cells from healthy mice were injected into mice that had received lethal doses of radiation (Ford *et al.* 1956; Till & McCulloch, 1961). The donor marrow was able to reconstitute all the differentiated blood cell types in the host and, when low numbers of cells were injected, colonies derived from single marrow cells (CFU-S) were found to contain both myeloid and erythroid cells (Wu *et al.* 1968). The use of chromosome markers induced by radiation or retroviral integration has confirmed that

cells of myeloid and lymphoid lineages can all be derived from a single cell with high self-renewal capacity (Abramson *et al.* 1977; Joyner *et al.* 1983; Williams *et al.* 1984; Dick *et al.* 1985; Lemischka *et al.* 1986).

The haemopoietic stem cells do not give rise to terminally differentiated cells directly, but *via* proliferating progenitor populations, as illustrated in Fig. 2. In this way, a relatively small number of stem cells (estimated as 0.4% of the total population of haemopoietic cells; Lord & Testa, 1988) can give rise to a large number of terminally differentiated cells (Lajtha, 1979). Identification of the progenitors and investigation of the mechanisms regulating their proliferation and differentiation have been made possible by the development of cell culture systems that support the proliferation of CFU-S and differentiation along multiple cell lineages (Pluznik & Sachs, 1965; Bradley & Metcalf, 1966; Dexter *et al.* 1977).

Although the existence of haemopoietic stem cells is well established, their identification has proved difficult. The strategy has been to separate subpopulations of marrow cells on the basis of buoyant density, sensitivity to antimetabolic agents or expression of cell surface antigens, and to look for enrichment of stem cells on the basis of *in vitro* or marrow reconstitution assays. In mouse, the following profile of the bone marrow reconstituting cells is emerging: they do not express any markers of either granulocyte, macrophage, B or T cell lineages (Müller-Sieburg *et al.* 1988).

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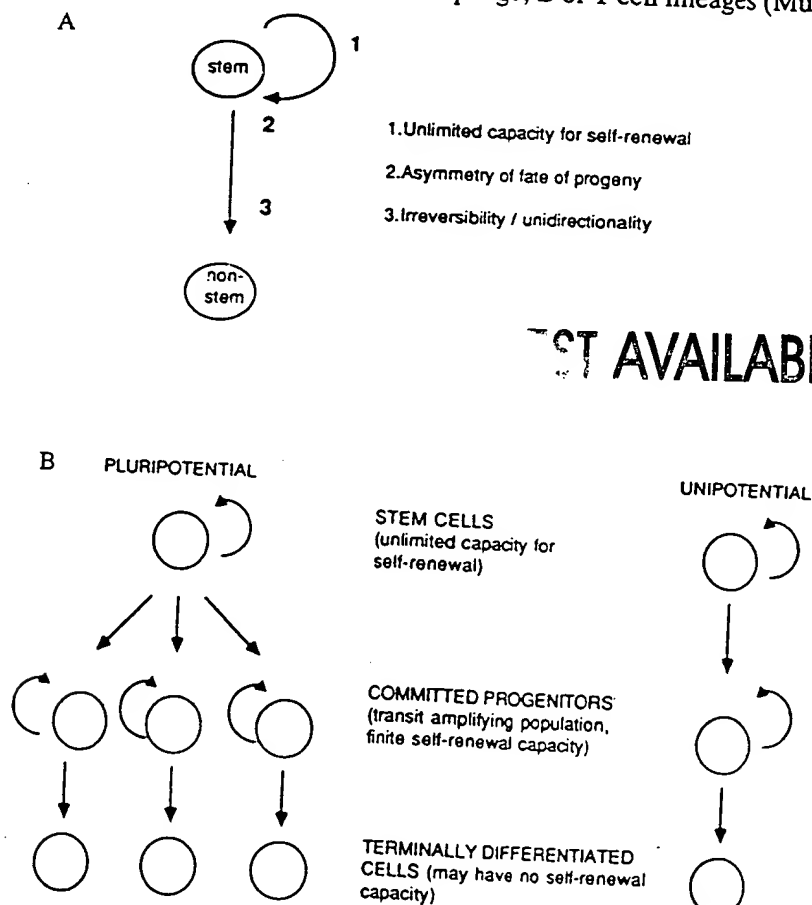


Fig. 1. Stem cell models.

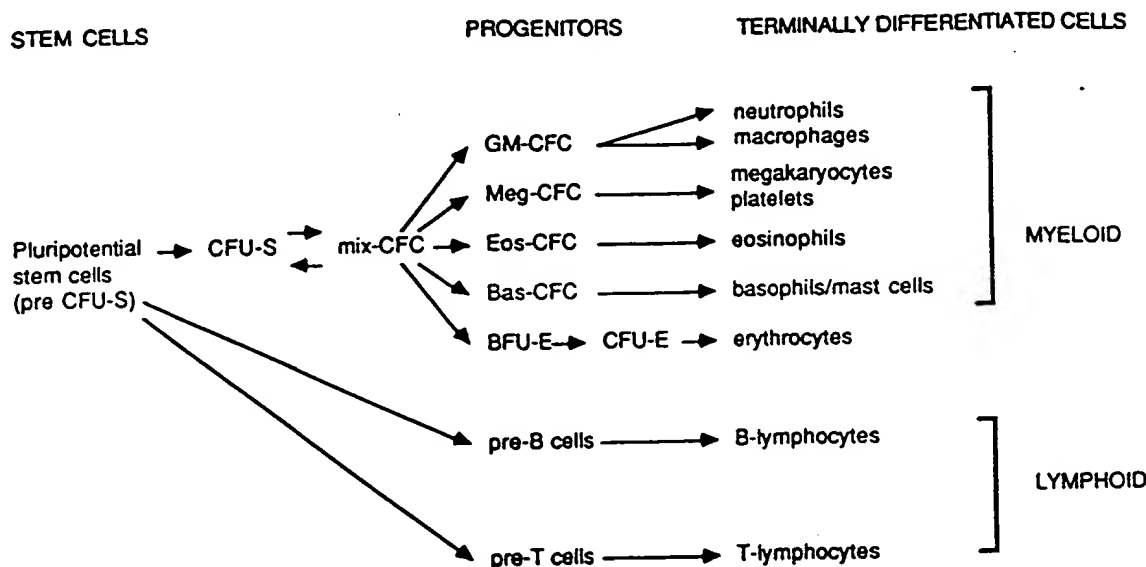


Fig. 2. The cells of haemopoietic tissue (redrawn from Dexter *et al.* (1985) with permission from Cambridge University Press). CFU-S: spleen colony-forming cells; mix-CFC: multipotential cells that can form colonies in soft agar; GM-CFC: granulocyte/macrophage colony-forming cells; Meg-CFC: megakaryocyte colony-forming cells; Eos-CFC: eosinophil colony-forming cells; Bas-CFC: basophil colony-forming cells; BFU-E: burst forming units-erythroid (primitive erythroid progenitors); CFU-E: colony forming units-erythroid (more mature erythroid progenitors).

but bind wheat germ agglutinin (Visser *et al.* 1984; Lord & Spooner, 1986), express low levels of Thy-1 (Müller-Sieburg *et al.* 1988) and express a surface antigen called SCA-1 (for Stem Cell Antigen 1) (Spangrude *et al.* 1988). In humans, 1–4 % of the marrow cells bear the CD34 antigen; these cells include virtually all the stem cells detected by *in vitro* assays, and baboon cells bearing CD34 are able to reconstitute the marrow of irradiated animals (Berenson *et al.* 1988). However, although subpopulations of bone marrow cells that contain haemopoietic stem cells can be defined, no unique markers of stem cell populations have yet been identified.

Cells within the bone marrow appear to lack the high degree of spatial organization characteristic of cells in epithelia. Nevertheless, studies of the distribution of different subpopulations of bone marrow cells suggest that it is not random (Western & Bainton, 1979; Lord & Testa, 1988). In mouse femur CFU-S with the highest self-renewal capacity, which are turning over very slowly, are found in the centre, while most CFU-S proliferation takes place in the vicinity of the bone. GM-CFCs are concentrated close to the bone surface, whereas maturing granulocytic cells accumulate towards the centre of the marrow space (Lord & Testa, 1988). A similar distribution has been reported in human rib (Testa, 1985).

Epidermis

The epidermis comprises multiple layers of epithelial cells, called keratinocytes (Fig. 3). The deepest layer, which is adherent to a basement membrane, contains most of the cells that are capable of dividing. Keratinocytes that leave the basal layer no longer divide and they undergo terminal differentiation as they move

towards the tissue surface. The end-point of the differentiation pathway is an anucleate, keratin-filled squame that is specialized to protect the underlying living cell layers from desiccation and mechanical damage. Squames are continuously shed from the surface of the epidermis and are replaced through proliferation of cells in the basal layer. In order that the epidermis remains a constant thickness, the rates of cell production and cell loss must be equal.

A number of observations suggest that not all of the keratinocytes that are capable of dividing are stem cells. For example, when mouse epidermis is severely damaged by radiation (Withers, 1967; Potten & Hendry, 1973) only about 10 % of the basal keratinocytes have sufficient proliferative potential to form recognizable foci of new epidermis. These clonogenic cells have been interpreted as constituting the stem cell population, whereas the further 50 % of basal cells that are capable of dividing may constitute a transit amplifying (progenitor) population. The remaining 40 % of cells in the basal layer are presumably postmitotic and committed to terminal differentiation (Iversen *et al.* 1968; Potten & Morris, 1988; Fig. 3).

Further evidence for proliferative heterogeneity has

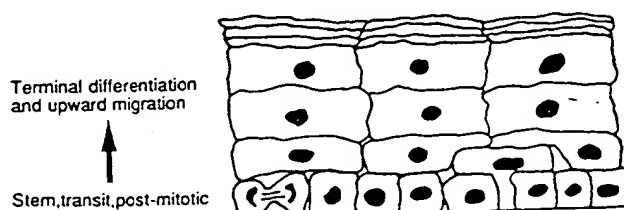


Fig. 3. Organization of keratinocytes in epidermis. Redrawn from Watt (1988), with permission. Copyright The Biochemical Society, London.

come from studies of cultured human epidermal keratinocytes. Sheets of cultured cells grafted onto suitable recipients form epidermis that persists for years, indicating that stem cells are not lost in culture (Gallico *et al.* 1984). However, only a small proportion of the cells in culture undergo extensive proliferation; the rest either fail to divide or form small abortive colonies in which all the cells terminally differentiate (Barrandon & Green, 1987b). Experiments in which the clone-forming ability of cells isolated from parental clones that differed in growth potential were examined indicate that transitions from clones of high growth potential to those with none occur in culture and are unidirectional (Barrandon & Green, 1987b; and see Fig. 1A).

Although extensive proliferation in culture or during recovery from radiation is thought to be characteristic of epidermal stem cells, there is evidence that under normal steady-state conditions the stem cells divide more slowly than the transit amplifying population. Thus kinetic analysis of mouse epidermis and *in vitro* experiments with cultured human keratinocytes suggest that stem cells have a longer cell cycle time and shorter S phase than transit amplifying cells, and that they retain [^3H]thymidine for much longer than the transit population (Bickenbach, 1981; Dover & Potten, 1983; Clausen *et al.* 1984; Jensen *et al.* 1985b; Morris *et al.* 1985; Albers *et al.* 1986). In addition, cells with stem-like characteristics (high capacity for self-renewal) are resistant to treatments that induce premature terminal differentiation in culture, such as exposure to TPA or cultivation in suspension (Parkinson & Emmerson, 1982; Parkinson *et al.* 1983; Hall & Watt, in preparation). However, although stem and transit amplifying keratinocytes can be distinguished by these functional criteria there are, to date, no molecular markers for each subpopulation.

In some stratified epithelia, there is evidence that the stem cells occupy a specific location within the basal layer. In the cornea, the stem cells appear to be located at the limbus (Schermer *et al.* 1986; Cotsarelis *et al.* 1989) and, in tongue papilla, they are found in those regions of the basal layer that project deeply into the underlying connective tissue (Hume & Potten, 1976). During wound healing, interfollicular epidermis can be regenerated from the outer root sheath of the hair follicles, suggesting that this is a source of stem cells (Lenoir *et al.* 1988). The basal layer in human and primate epidermis is not flat, but undulates, and there is some evidence that the stem cells – identified as slowly cycling cells – are located in the troughs (Lavker & Sun, 1983).

In some regions, such as the dorsal skin of the mouse, the basal layer is flat and keratinocytes in the suprabasal layers are arranged in columns, like a stack of coins (Mackenzie, 1970; Christophers, 1971). It has been proposed that each stack corresponds to an epidermal proliferative unit (EPU), consisting, in the basal layer, of a stem cell surrounded by 5–6 transit amplifying cells and less than four postmitotic cells (i.e. committed to terminal differentiation) (Potten, 1974, 1981). The

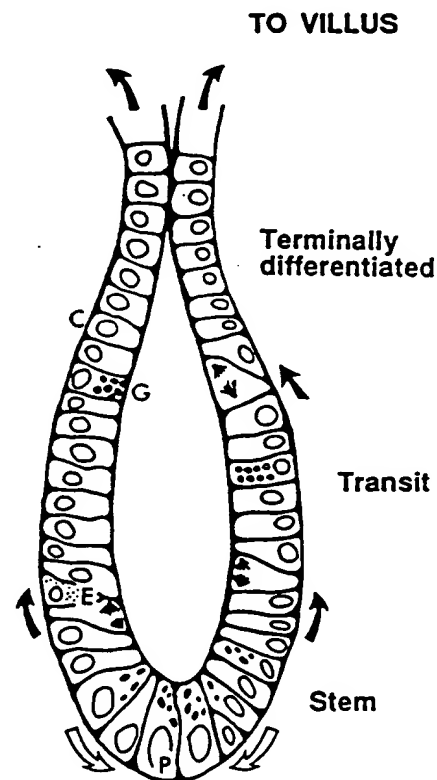


Fig. 4. Organization of cells in the crypt of the small intestine. Redrawn from Potten (1983), with permission from Churchill-Livingstone. C: columnar cell; G: goblet cell; E: entero-endocrine cell; P: Paneth cell.

model is based on cell kinetic analysis and is supported by the findings that the basal cells that retain [^3H]thymidine (putative stem cells – see earlier) lie at the centre of the stacks of suprabasal cells in mouse dorsal epidermis (Morris *et al.* 1985) and that gap junctional communication compartments are approximately the same size as EPUs (Pitts *et al.* 1988). However, a columnar arrangement of keratinocytes is lacking in most body sites, and the pattern of mosaicism revealed in the epidermis of chimaeric mice is not consistent with the EPU model (Schmidt *et al.* 1987).

Gastrointestinal epithelium

The lining of the small intestine is a monolayer of epithelial cells organized into crypts and villi (Fig. 4). Cell proliferation is confined to the crypts; differentiated cells migrate to the surface and are shed from the tips of the villi. There are four differentiated cell types: Paneth cells at the base of the crypts and columnar, goblet and entero-endocrine cells in the rest of the crypts and on the villi; none of these mature cells divide, suggesting that they are renewed by proliferation of a stem cell population. Evidence for the existence of a single stem cell compartment generating all four types of progeny came first from the observation that, after radiation-induced cell death, surviving proliferative cells phagocytose cell debris and the phagosomes can be used as markers of their progeny: within 30 h phagosomes are found in all the differentiated cell

types (Cheng & Leblond, 1974). Further evidence that intestinal stem cells are pluripotential comes from the finding that lines derived from single human adenocarcinoma cells can differentiate into columnar, goblet and entero-endocrine cells in culture (Huet *et al.* 1987; Kirkland, 1988).

The stem cells are thought to be located at or near the base of each crypt, since proliferating cells higher in the crypt migrate upwards and are not therefore permanent residents (Potten *et al.* 1987). It has been proposed that stem cells give rise to committed progenitors for each differentiated cell type (Cheng & Leblond, 1974; see Fig. 4). Kinetic studies suggest that there are 4–16 stem cells per crypt (Potten & Loeffler, 1987; Potten *et al.* 1987). However, studies with mouse aggregation chimeras or mice heterozygous for markers that can be detected histochemically show that the epithelium of individual crypts in the small and large intestine of adult mice is always composed of cells of a single parental type, and hence that each crypt is probably derived from (and maintained by) a single stem cell during development (Ponder *et al.* 1985; Griffiths *et al.* 1988; Schmidt *et al.* 1988; Winton *et al.* 1988). In adult colonic epithelium there is evidence that each crypt is maintained by proliferation of one stem cell (Griffiths *et al.* 1988; Winton *et al.* 1988; Fig. 5). No molecular markers for intestinal stem cells have so far been reported.

General characteristics of stem cells

In the previous sections, we described evidence for the existence of stem cells in bone marrow, epidermis and intestinal epithelium, and what progress has been made towards their identification. In all three tissues, a relatively small number of stem cells give rise to a large number of terminally differentiated cells *via* a transit



Fig. 5. Section of human colon stained with an antibody that recognizes acetyl groups on O-linked carbohydrate chains of mucins. Cells in most crypts express the enzyme, but rare crypts (3 shown here) are unstained. Analysis of this pattern supports the conclusions from mouse studies (see text) that each crypt can be founded by a single stem cell (P. Richman, unpublished observations). Bar = 100 μ m. Photograph kindly provided by P. Richman.

amplifying (progenitor) population and the stem cells differ from their progeny in phenotype and/or location. In each tissue, the processes of stem cell renewal and production of differentiated progeny must be tightly coordinated, in order to meet the varying requirements of the body for differentiated cells. These common features enable us to ask a number of general questions about stem cells:-

1. What mechanisms determine asymmetry of fate, such that stem cells produce both stem cells and daughters committed to terminal differentiation?
2. What mechanism allows for unlimited self-renewal of stem cells, but a finite number of rounds of division within the transit/progenitor populations?
3. Is differentiation from the stem cell compartment irreversible?

Observations from a range of different tissues and organisms provide partial answers to these questions.

Asymmetric divisions

One feature of all three stem cell systems is their capacity to produce both stem cells and cells that are committed to terminal differentiation, represented symbolically as $A \rightarrow A + B$. This can, theoretically, be achieved in two ways. In the first, the outcome of every division is predetermined and invariant: $A \rightarrow A + B$. In the second, individual stem cell divisions may have different outcomes, such that $A \rightarrow A + A$ or $B + B$ or $A + B$, but, on a population basis, the end result is the production of equal numbers of A and B. This second situation could arise in three ways: if the outcome of each division is stochastic; if it is environmentally regulated; or if the stem cell population is heterogeneous.

Some of the best examples of invariant asymmetric divisions are found in yeast, *Caenorhabditis elegans* and plants, in which lineage maps of cell fate can be constructed. In cases where lineage is not the sole determinant of the outcome of divisions, such as in adult mammalian tissues, in *Hydra* and certain *C. elegans* tissues, some progress has been made in identifying regulatory factors.

Predetermined asymmetry

Yeast

Yeast cells can either be haploid or diploid, the diploid state resulting from fusion of two haploid cells that differ in mating type. Although the progeny of haploid cells normally inherit the mating type of the parental cell, switching of mating type can occur. It is the ability to switch that is inherited asymmetrically (Strathern & Herskowitz, 1979; Nasmyth, 1983; Klar, 1987a; Nasmyth & Shore, 1987).

In the budding yeast, *Saccharomyces cerevisiae*, only the mother cells, and not their daughters (the buds), have the ability to switch mating type. Mating type is determined by gene conversion at a specific locus,

which occurs through the action of a specific endonuclease, encoded by the *HO* gene, such that the gene conferring one mating type is excised and replaced with a copy of the gene conferring the other type (Hicks & Herskowitz, 1976). *HO* expression and mating-type switching are restricted to a short period in late G_1 , after the cell has become committed to another mitotic cycle (Nasmyth, 1983). *HO* transcription is controlled both positively and negatively by the products of several other genes, at least one of which interacts directly with the *HO* promoter (Nasmyth *et al.* 1987; Sternberg *et al.* 1987). The ability of mother, but not daughter, cells to transcribe *HO* appears to be due to asymmetric distribution of these gene products at division (Klar, 1987b).

In the fission yeast, *S. pombe*, mating type switching occurs such that of the four progeny of a single cell obtained after two generations, only one is switched; in other words, only one of the two daughters in the first generation is competent to produce a switched cell in the subsequent generation. As in *S. cerevisiae*, mating type is determined by gene conversion but, in *S. pombe*, the ability to switch is inherited chromosomally, not through unequal distribution of cytoplasmic or nuclear factors (Egel, 1984; Klar, 1987a,b). Current models suggest that asymmetry results from inheritance of nonequivalent parental DNA molecules (Klar, 1987a,b).

Caenorhabditis elegans

C. elegans development occurs via an essentially invariant sequence of cell divisions and lineage maps of the origin of every cell in the adult organism can be constructed (Sulston, 1988). There is some evidence that stem-cell-like lineages underlie normal development. For example, in the V lineages of the lateral hypoderm, each seam cell divides repeatedly to generate one seam cell and one syncytial cell (Sulston & Horvitz, 1977). Similarly, the original zygote divides to produce one totipotent cell at each division that eventually generates a single cell that is solely responsible for producing the entire germ line (Sulston *et al.* 1983).

A major step towards understanding the genetic basis of *C. elegans* cell lineage has been the identification and characterization of mutations in the genes involved in causing sister cells, or mother and daughters, to have different fates (Horvitz, 1988). For example, in many of the lineages affected by *unc-86* mutations one of the two daughters of a division fails to express its normal fate and retains the fate of its own mother cell; this results in a stem-cell-like lineage (Chalfie *et al.* 1981). *lin-17* mutations result in both sister cells expressing the same fate (Sternberg & Horvitz, 1988), and, in *lin-14* mutations, certain cells express fates that would ordinarily be expressed earlier or later in larval development (Ambros & Horvitz, 1984, 1987).

What are the mechanisms of predetermined asymmetric divisions?

S. cerevisiae and *S. pombe* provide examples of two possible mechanisms for producing daughters that dif-

fer in fate: either that some determining factor (cytoplasmic or nuclear) is inherited asymmetrically or that the parental DNA strands are different (nonequivalent). In *S. cerevisiae* unequal partitioning of the factors that regulate *HO* transcription may occur at mitosis (Klar, 1987b). Possible mechanisms resulting in nonequivalence of DNA strands include asymmetric methylation of specific loci (Monk, 1988) or asymmetric binding of transcription factors (Miller *et al.* 1985; Weintraub, 1985). There is circumstantial evidence for genomic imprinting in *Aspergillus* (Rosenburger & Kessel, 1968) and the stem cells of plant meristems such as *Zea mays* (Barlow, 1978). However, in early *C. elegans* development the parental DNA strands segregate randomly (Ito & McGhee, 1987).

Nonequivalence of DNA strands is a central feature of the immortal strand hypothesis of Cairns (1975), which proposes that, during an asymmetric stem cell division, the new stem cell always retains the parental DNA strand, while the daughter that is destined to differentiate inherits the newly synthesized strand. This would provide a means for protecting stem cell DNA from errors during the replication process. [3 H]thymidine labelling patterns in mouse intestinal crypts and tongue papillae are consistent with the hypothesis (Potten *et al.* 1978), but it remains to be proven.

One final mechanism that might ensure asymmetry of fate of stem cell progeny would be for spatial location to determine fate. The cell that retained the position of the parental cell would itself be a stem cell, while the other cell would be committed to differentiate. This, however, implies environmental regulation of cell fate (see later) and would only constitute a predetermined outcome in the sense that the relative positions of the two daughters are fixed; for example, in the root-tip of the water fern *Azolla* (Gunning *et al.* 1978).

Asymmetry without lineage involvement

Stochastic events

One approach to investigate whether the outcome of stem cell divisions is stochastic is to isolate individual stem cells, place them in culture under identical conditions, and analyse the fate of their progeny. If the stem cells are all equivalent yet found colonies containing different proportions of self-renewing and differentiating progeny, and different types of differentiated progeny, this would argue that fate is acquired randomly. Experiments that address this question in the haemopoietic system have been carried out by Johnson & Metcalf (1977, 1979) and Suda *et al.* (1984). The liver is a site of haemopoiesis in foetal mice and individual cells from this tissue are able to form colonies containing, in addition to erythroid cells, macrophages, neutrophils, megakaryocytes or eosinophils (Johnson & Metcalf, 1977). Single cells isolated from fractionated foetal peripheral blood were grown to clones of 3–8 cells: individual cells were then cultured separately and the differentiation status of their clones assayed after several days. The experiments showed that within the first

three divisions of a haemopoietic stem cell and its progeny, at least one cell with pluripotentiality can be produced and at least two committed progeny can arise from the two or three divisions (Johnson & Metcalf, 1979). Similarly, Suda *et al.* (1984) demonstrated that the two daughters of a CFU-S can differentiate along different lineages. Since it must be assumed that the cellular environment was the same for each cell, the experiments show that self-renewal and commitment can occur in the absence of a directive environmental influence.

Stem cell heterogeneity

Another mechanism that would ensure that stem cell divisions produce both stem cells and differentiated cells would be if the stem cell population is heterogeneous, with some stem cells having a high probability of self-renewal and others a high probability of differentiation. There is evidence for heterogeneity of haemopoietic stem cells in their ability to self-renew and repopulate irradiated bone marrow (Goodman *et al.* 1977; Hellman *et al.* 1978; Harrison *et al.* 1987a). This has led to the proposal that the stem and transit populations are not discrete (as shown in Fig. 2), but represent a continuum from cells of high self-renewal capacity and low probability of differentiation to those of low self-renewal capacity and high commitment to differentiation (Goodman *et al.* 1977; Hellman *et al.* 1978; Brown *et al.* 1989).

One source of heterogeneity could be stem cell aging. This has not been observed for haemopoietic cells within the lifetime of individual animals, since the repopulating capacity of foetal, young or old mouse marrow is the same (Lajtha & Schofield, 1971; Harrison *et al.* 1978, 1984). However, there is a decrease in self-renewal capacity with serial transfer of murine haemopoietic stem cells between animals (Siminovitch *et al.* 1964; Cudkowicz *et al.* 1964; Harrison, 1973; Harrison & Astle, 1982; Ogden & Micklem, 1976). Barrandon & Green (1987b) have observed a decrease in the proportion of human keratinocytes capable of sustained self-renewal in culture with increasing donor age.

The clonal succession model of Kay (1965) proposes that there is sequential activation of stem cells during the lifespan of an organism. This is supported by the observations of Lemischka *et al.* (1986) and Mintz *et al.* (1984). Lemischka *et al.* used retrovirus-mediated gene transfer to mark haemopoietic stem cells and follow their fate after transplantation into lethally irradiated recipients. In most cases, 1 or 2 stem cell clones accounted for the majority of the mature haemopoietic cells at any one time, and different clones were active at different times in the life of the animals. Mintz *et al.* used a mixture of haemopoietic stem cells from two normal mice strains to repopulate mouse foetuses from a third strain with a defect in haemopoiesis. In some mice, there was a regular and complementary rise and fall in the proportion of haemopoietic cells of the two normal genotypes, thus supporting the clonal succession model. However, Harrison *et al.* (1987b, 1988), also studying haemopoiesis in chimaeric mice, have

obtained evidence that many stem cells are active simultaneously, and the reason for the discrepancy is unclear.

Environmental regulation

Although development of *C. elegans* is largely invariant, there is good evidence for the existence of small groups of cells, known as equivalence groups, which arise from very similar lineages and share the same potential. In such groups, cell interactions result in member cells following different developmental pathways and, if these interactions are perturbed experimentally, the differentiation fate of the cells is altered. Two examples of this are vulval development (Sternberg & Horvitz, 1986; Sternberg, 1988) and the regulation of meiosis in the adult germ line (Austin & Kimble, 1987). In the vulva, the fate of each precursor cell depends on a combination of two intercellular signals: an inductive signal from the anchor cell and a lateral inhibitory signal from neighbouring precursor cells. Mutations in the *lin-12* locus affect vulval development (Greenwald *et al.* 1983) and this gene encodes a putative transmembrane receptor with epidermal growth factor-like repeat sequences (Yochem *et al.* 1988). In the germ-line, interaction with a somatic cell, the distal tip cell, is required for continued mitosis; if the distal tip cell is removed, all germ cells enter meiosis and form gametes. The product of a wild-type gene *glp-1* is required for germ cell mitoses. In mutants of *glp-1*, the distal tip cell is present, but there is a defect in the response of cells to the signal: the defect appears to involve a transmembrane receptor (Austin & Kimble, 1987).

In *Hydra* all the differentiated somatic cell types and the male and female gametes are derived from a population of multipotent interstitial stem cells which have an extensive capacity for self renewal (Bosch & David, 1987; David *et al.* 1987). The number of the stem cells in different body regions differs: most are found in the stalk with few in the arms or foot. In *Hydra*, environment rather than lineage plays the major role in determining the fate of stem cell progeny: spatial variation in the distribution of different differentiated cell types is a consequence of local commitment following stem cell migration. A peptide called head activator has been isolated which is secreted by nerve cells and causes stem cells to differentiate into nerve cells (Schaller & Bodenmuller, 1981). Head activator is required for head, as opposed to foot, growth and differentiation. In addition, there is an inhibitory activity originating in the head and preventing head formation elsewhere in the body - this inhibitory effect is perturbed when gap junctional communication is blocked (Fraser *et al.* 1987).

A further example of environmental influence on stem cell behaviour in *Hydra* is the mechanism ensuring that the ratio of stem cells to epithelial cells is constant. If the number of interstitial stem cells is experimentally reduced, there is a rapid increase in the size of the stem

cell population until the normal stem cell density is achieved. This is achieved by an increase in the probability of self-renewal of the stem cells (David *et al.* 1987). Similar homeostatic mechanisms presumably maintain stem cell density in other organisms, but they have still to be identified.

The studies on *Hydra* and on *C. elegans* equivalence groups point to the importance of cell-cell interactions involving diffusible signals. In haemopoietic tissues and epidermis, there is also strong evidence that diffusible molecules, specifically growth factors, regulate stem cell behaviour.

A number of different growth factors, produced by the marrow stroma (and other cell types), are required for haemopoietic cell survival, differentiation and proliferation (Clark & Kamen, 1987; Dexter & Spooner, 1987; Sachs, 1987; Metcalf, 1989). They include the major myeloid growth factors interleukin 3, granulocyte-macrophage colony-stimulating factor (CSF), granulocyte CSF and macrophage CSF. They act at different levels in the differentiation pathway and have different target cells, some acting on multiple lineages while others are restricted to a single lineage. Interactions between different factors maintain the correct balance between the different differentiated cell types and between stem cells, progenitors and terminally differentiated cells (Heyworth *et al.* 1988). Each growth factor binds to specific receptors, and the binding of a growth factor to its receptor can lead to down-regulation of expression of receptors for other growth factors (Walker *et al.* 1985). Stem cells appear to have receptors for all the growth factors and there is selective loss of particular receptors as their progeny become committed progenitors (Heyworth *et al.* 1988). Factors that inhibit proliferation of haemopoietic stem cells have also been reported (Lord *et al.* 1976; Lenfant *et al.* 1989).

As in haemopoietic tissue, there is evidence that proliferation of epidermal cells is controlled by growth stimulatory and inhibitory factors (reviewed by Watt, 1988). EGF and TGF α increase the number of cell generations in cultures of human keratinocytes without increasing the growth rate (Rheinwald & Green, 1977) and stimulate lateral migration of proliferative cells at the periphery of expanding colonies (Barrandon & Green, 1987a). TGF β inhibits proliferation and causes growth arrest in G₁ (Shipley *et al.* 1986). Whereas in haemopoiesis the target cells for different growth factors have been defined, it is unclear in the epidermis whether any of the growth regulatory molecules show specificity towards stem or transit amplifying cells (Jensen *et al.* 1985a). However, it is known that keratinocytes synthesize molecules that regulate their own growth (e.g. TGF α and β) and that the level of expression can change in response to environmental stimuli (Coffey *et al.* 1987; Akhurst *et al.* 1988). It has also been proposed that gap junctional communication may play a role in epidermal growth regulation (Pitts *et al.* 1988). Less is known about the effect of growth regulatory molecules on intestinal epithelial cells, but it has recently been shown that TGF β is produced by

terminally differentiated cells at the tip of the villus and inhibits crypt cell proliferation (Barnard *et al.* 1989).

Diffusible molecules are not the only factors that regulate stem cell behaviour, and evidence is accumulating for the importance of the extracellular matrix. In bone marrow the haemopoietic stem cells and progenitors are in intimate contact with stromal cells. In long term cultures of bone marrow, the feeder layer of stromal cells (Fig. 6) can support haemopoiesis even when glutaraldehyde-fixed (Roberts *et al.* 1987) and can be replaced by a combination of extracellular matrix proteins and growth factors. Heparan sulphate in the matrix specifically binds erythropoietin and IL-3 and the bound growth factors retain biological activity (Gordon *et al.* 1988; Roberts *et al.* 1988). The observations that growth factors can regulate production of extracellular matrix components (e.g. IL-3 stimulates synthesis of a human eosinophil proteoglycan; Rothenberg *et al.* 1988) and that cells at different stages of haemopoietic differentiation differ in adhesiveness to the marrow stroma (reviewed by Gordon, 1988) point to synergism between extracellular matrix molecules and growth factors.

One interesting experimental model for investigating interactions between haemopoietic stem cells and the marrow stroma is provided by two mutant mouse strains that both develop macrocytic anaemia (McCulloch *et al.* 1964, 1965). Lesions in *W/W* mice are thought to be due to intrinsic defects in the haemopoietic cells, whereas in *Sl/Sl* mice the defect is in the haemopoietic microenvironment. If *W* stroma is combined with *Sl* marrow in culture there is sustained production of differentiated haematopoietic cells, but *Sl* stroma plus *W* stem cells (or indeed, *W + W* or *Sl + Sl*) does not (Dexter & Moore, 1977). The *W* locus encodes a transmembrane receptor with tyrosine kinase activity (*c-kit*) and it has been proposed that its ligand, possibly an extracellular matrix component (Morrison-Graham & Weston, 1989), might be encoded by the *Sl* locus (Geissler *et al.* 1988; Chabot *et al.* 1988). The presence of abnormalities in other tissues such as melanocytes and testis suggest that the *c-kit* gene and its ligand may be important in the control of proliferation and differentiation in other stem cell systems.

As in the bone marrow stroma, there is evidence that interactions between epithelial cells and the extracellular matrix can regulate proliferation and terminal differentiation (Bissell *et al.* 1982; Watt, 1986). Thus, in cell lines derived from human colon carcinomas, the ability to undergo morphological differentiation is correlated with the ability to adhere to collagen (Richman & Bodmer, 1988; Pignatelli & Bodmer, 1988). During epidermal terminal differentiation keratinocytes develop reduced adhesiveness to basement membrane components (reviewed by Watt, 1987), and fibronectin can inhibit suspension-induced terminal differentiation (Adams & Watt, in preparation). It is quite possible that ECM-growth factor interactions are important in regulating epidermal, as well as haemopoietic, proliferation, since, for example, fibronectin has been shown to bind TGF- β (Fava & McClure, 1987).



Fig. 6. Scanning electron micrograph of a long term mouse bone marrow culture, showing adhesion of early monocyte/macrophages to fixed bone marrow stromal cells (the large, well-spread cells) (see Roberts *et al.* 1987). Bar = 50 μ m. Photograph kindly provided by T. D. Allen.

Conclusions: the 'niche' hypothesis

In 1967 Curry and Trentin proposed that the differentiation pathways taken by the progeny of haemopoietic stem cells are determined by different haemopoietic inductive microenvironments within the spleen and bone marrow. This model has been extended by the 'niche' hypothesis of Schofield (1978), which proposes that a stem cell resides in an optimal microenvironment, or niche. When such a cell divides only one daughter can remain in the niche and the other will be committed to differentiate unless another niche is available for it. A stem cell within the niche would have a high probability of self-renewal, but low probability of entry into the cell cycle and would thus divide only rarely. It is possible that different sites within the microenvironment would have differing degrees of 'niche-ness', resulting in stem cells with differing probabilities of self-renewal, in other words, a hierarchy of cells with an increasing probability of undergoing differentiation. This would fit with the observed heterogeneity of the haemopoietic stem cell population with respect to self-renewal capacity and ability to repopulate marrow-depleted hosts and, since the model is probabilistic, it also allows for stochastic events.

From our description of environmental factors that regulate stem cell behaviour, we can assume that major components of the stem cell microenvironment will be the other cell types present (e.g. the anchor and distal tip cells in *C. elegans*; bone marrow stromal cells); diffusible factors; and extracellular matrix molecules.

None of these can be considered in isolation, however, since different cell types secrete different growth factors and lay down extracellular matrices that differ in composition (Watt, 1986); growth factors regulate extracellular matrix synthesis and breakdown (Sporn *et al.* 1987); and the extracellular matrix can bind specific growth factors, thus increasing their local concentration (Gordon *et al.* 1988; Roberts *et al.* 1988).

What is the difference between two daughter cells that result from an asymmetric division?

In the previous section, we discussed the mechanisms by which two daughters of a stem cell division could have different fates. In this section, we shall briefly consider what is known of the genes determining cell fate. There is good evidence from experiments involving nuclear transplantation, cell fusion and transdetermination that differentiation does not involve loss or irreversible repression of genes (Gurdon, 1962; Yamada & McDevitt, 1974; Blau *et al.* 1983). Instead, current models suggest that differentiation leads to activation of a small number of 'master' genes that in turn control a regulatory cascade, resulting in the overall pattern of gene expression characteristic of a given cell type (Gierer, 1973; Blau, 1988; Maniatis *et al.* 1987).

A good illustration, from *C. elegans*, of such a 'master' gene is *unc-86* which is required for generation

of the neural cell lineages that generate mechanosensory receptors. In *unc-86* mutants, the neuroblasts undergo simple stem cell divisions, since the normal gene product is required to change a cell from the state of the mother to the daughter cell (Chalfie *et al.* 1981). *Unc-86* encodes a transcription factor, which is proposed to control expression of a number of other genes that determine cell fate (Finney *et al.* 1988). *Unc-86* shares a large region of homology with at least three mammalian proteins that regulate transcription, two of which, Pit-1 and Oct-2, regulate cell-specific genes in pituitary cells and B-lymphocytes, respectively (Herr *et al.* 1988).

Another situation in which genes that regulate differentiation have been identified is muscle. Treatment of a mouse embryonic cell line, C3H10T^{1/2}, with 5-azacytidine leads to formation of clones of myoblasts, adipocytes and chondrocytes, suggesting that 10T^{1/2} cells contain a multipotential progenitor population and that differentiation requires demethylation of genes (Taylor & Jones, 1979). Transfection of 10T^{1/2} cells with a human gene called *myd* results in commitment to myogenesis (Pinney *et al.* 1988) and subsequent expression of MyoD1 results in expression of muscle-specific genes (Davis *et al.* 1987; Tapscott *et al.* 1988). Interestingly, when 10T^{1/2} cells are transfected with the muscle-specific gene troponin 1, there is appropriate expression only during myoblast differentiation, not in uncommitted cells (Konieczny & Emerson, 1985). MyoD1 is a nuclear phosphoprotein that has homology with *myc* and can bind DNA (Tapscott *et al.* 1988; Murre *et al.* 1989); it is normally expressed only in mouse skeletal muscle *in vivo* and myogenic cell lines *in vitro*. Recently a number of other genes with homology to MyoD1 have been identified, suggesting that it is a member of a small gene family that regulates myogenesis (Wright *et al.* 1989; Braun *et al.* 1989).

Given that a small number of 'master' genes encoding specific DNA-binding proteins may control expression of those genes that confer the differentiated phenotype of a cell, one important question remains: what regulates expression of the master genes? There is good evidence (see previous section) that environmental factors can determine the fate of stem cell progeny, and so it is likely that they can activate the 'master' genes. In support of this, there is preliminary evidence that MyoD1 expression may be regulated by exogenous mitogens and by cell density (Tapscott *et al.* 1988).

Unlimited self-renewal versus finite transit amplifying divisions

Stem cells, by definition, have an extensive capacity for self-renewal that extends throughout the life of the organism and so is, essentially, unlimited. In contrast, there is good evidence from a number of cell culture models that the number of rounds of division that a transit amplifying cell can undergo is finite. Transit amplifying keratinocytes undergo terminal differentiation within 15 cell generations in culture (Barrandon

& Green, 1987b). The 0-2A progenitor cells of rat optic nerve undergo a finite number of divisions in the presence of platelet-derived growth factor (PDGF) produced by type 1 astrocytes before differentiating into oligodendrocytes (Temple & Raff, 1986; Raff *et al.* 1988). Quinn *et al.* (1985, 1988) have evidence for the existence of a stem cell compartment in skeletal muscle that generates committed precursors which undergo four determined, symmetric divisions to produce 16 terminally differentiated, postmitotic skeletal muscle cells (note, however, that other studies do not support this conclusion; Konigsberg & Pfister, 1986).

The mechanism that determines the number of divisions in these situations is unknown, but one possibility is that the cells are able to count cell divisions and after the appropriate number undergo terminal differentiation. In 0-2A cells, it has been proposed that a stable molecule required for proliferation in response to PDGF is diluted with each cell division and when its concentration falls below a certain level the cells stop dividing and differentiate (Temple & Raff, 1986; Raff *et al.* 1988). PDGF receptors are still present on the surface of newly differentiated oligodendrocytes, so receptor loss is not the reason for cessation of cell division (Hart *et al.* 1989).

The differentiation process is unidirectional

When the product of terminal differentiation is an anucleate cell, such as an erythrocyte or an epidermal squame, it is obvious that it cannot give rise to a stem cell and reverse the differentiation process. However, at the other end of the spectrum, in yeast, a switch in mating type can be reversed in subsequent generations (Klar, 1987a). In most experimental situations, there is evidence for progressive restriction in cell fate: good examples are human keratinocytes (Barrandon & Green, 1987b) and progenitor cells for different mesenchymal cell types (Grigoriadis *et al.* 1988).

The mechanisms that result in progressive restriction of cell fate are not clear. Holtzer (1978) has proposed that it requires 'quantal cell cycles' by which daughters acquire a different phenotype from their mother. However, both muscle cells (Clegg *et al.* 1987) and keratinocytes (Parkinson *et al.* 1983; Hall & Watt, in preparation) can undergo terminal differentiation without an intervening round of mitosis, through mitogen withdrawal or suspension, respectively. Brown *et al.* (1989) argue that lineage options are programmed within cells, and differentiation from the stem cell compartment is unidirectional because lineage potentials are only available in a predetermined sequence.

A different model would propose that unidirectionality is ensured by departure from the stem cell niche, since changes in cell phenotype as part of the differentiation process might normally ensure that the differentiating cell cannot re-enter, or respond to, the niche microenvironment. For example, overt terminal differentiation of muscle cells (Clegg *et al.* 1987) and haemopoietic stem cell progeny (Heyworth *et al.* 1988)

is preceded by loss of growth factor receptors and there is a reduction in cell-ECM adhesiveness during terminal differentiation of haemopoietic (Mauch *et al.* 1980; Kerk *et al.* 1985; Gordon *et al.* 1987; Gordon, 1988) and epidermal cells (reviewed by Watt, 1987; Adams & Watt, in preparation).

Although differentiation from the stem cell compartment is normally unidirectional, it can be reversed under some experimental conditions. For example, E1a infection of keratinocytes that are committed to terminal differentiation can rescue them from abortive colony formation, resulting in immortalized cell lines (Barrandon *et al.* 1989). Differentiated plant cells are remarkable in that they generally remain pluripotent and, in response to a suitable stimulus, can divide to give rise to a range of other differentiated cell types (Steward *et al.* 1964).

The most promising approach for discovering the molecular basis of unidirectionality is at present in *C. elegans*, where a series of heterochronic mutations alter the relative timing of different developmental events (Ambros & Horvitz, 1984). For example, the *lin-14* locus plays a major role in determining the fate of cells in several different tissues and lineages: high levels of *lin-14* protein specify cell fates characteristic of early developmental stages and low levels specify late stages (Ambros & Horvitz, 1987; Ruvkun & Giusto, 1989). Most of the heterochronic genes, including *lin-14*, are pleiotropic, but those (such as *lin-29*, which only affects lineages of the lateral hypodermis at one developmental stage) with tissue- or stage-specific effects may be required for response to the temporal information imparted by the others (Ambros & Horvitz, 1984). Heterochronic mutations in mammalian species are likely to be lethal early in development, and none have been reported to date.

Conclusions and prospects

Stem cells are responsible for the generation and maintenance of terminally differentiated cell populations in tissues that undergo continuous turnover. In this review, we have described some characteristics of stem cells and what is known of the mechanisms regulating their behaviour. The molecular basis for asymmetric divisions and specification of cell fate is much better understood in lower organisms, than in mammalian tissues, but we believe that similar regulatory mechanisms may operate in both. In mammalian systems, there is a clear need for markers to distinguish stem from transit cells (if, indeed, markers exist). Cell culture models of haemopoietic, epidermal and intestinal differentiation can be exploited to study the determinants of asymmetry at the level of individual mitoses. It is also important to identify potential cascades of gene expression that specify particular differentiation pathways, and investigate the extent to which these events can be reversed. Finally, the information obtained from studies of stem cells in normal tissues may shed some light on a variety of pathological conditions,

in particular metaplasia (in which reprogramming of stem cells may cause their progeny to convert from one differentiation pathway to another, Slack, 1986) and neoplasia (in which the normal balance between proliferation and differentiation is perturbed; Cairns, 1975; Buick & Pollack, 1984; Pierce & Speers, 1988; Anon, 1989).

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